Integration of Nonchemical Treatments for Control of Postharvest Pyralid Moths (Lepidoptera: Pyralidae) in Almonds and Raisins

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ABSTRACT We propose a treatment strategy combining an initial disinfestation treatment with one of three protective treatments as an alternative for chemical fumigation of almonds and raisins for control of postharvest insect populations. Initial disinfestation treatments using low oxygen controlled atmosphere (0.4% O_2) were designed to disinfest product of field populations of pyralid moths; navel orangeworm, *Amyelois transitella* (Walker), in almonds and raisin moth, *Cadra figulilella* (Gregson), in raisins. The protective treatments were cold storage (10°C), controlled atmosphere (5% O_2) storage, and application of the Indianmeal moth granulosis virus, and were designed to prevent establishment of Indianmeal moth, *Plodia interpunctella* (Hübner). The initial disinfestation treatment was effective against laboratory populations of navel orangeworm and raisin moth. Efficacy of protective treatments was determined by exposure of commodities to laboratory Indianmeal moth populations at levels far higher than those found in commercial storage facilities. All three protective treatments prevented development of damaging Indianmeal moth populations as measured by pheromone trap catches and evaluation of product samples. Quality analysis by commercial laboratories showed that overall product quality for all protective treatments was maintained at levels acceptable by industry standards.

KEY WORDS *Cadra figulilella, Amyelois transitella, Plodia interpunctella,* controlled atmospheres, cold storage, granulosis virus

POSTHARVEST INSECTS CAUSE losses to dried fruits and nuts during storage through direct damage, product contamination, and creation of favorable conditions for mold growth and product degradation. In California, where the annual production of dried fruits and nuts is >one million metric tons and is worth nearly $1.5 billion U.S. (USDA 1998), costs due to insect-related product loss and control measures are substantial. Currently, these industries depend on fumigation with methyl bromide or phosphine for postharvest insect control. Processors use fumigants to disinfest large volumes of the incoming product during harvest, and to control infestations throughout storage durations that may exceed a year.

After action taken in 1992 by >100 signatory nations of the Montreal Protocol, methyl bromide was designated an ozone depleter (UNEP 1992). Although the U.S. Clean Air Act would have eliminated production and importation of methyl bromide in this country by 1 January 2001, recent legislation brought the U.S. phaseout of methyl bromide in line with that of the Montreal Protocol, with near complete reduction scheduled for 2005. Insect resistance to hydrogen phosphine has been documented in other commodities (Zettler et al. 1989), and the USEPA is considering increased restrictions on the use of this fumigant (USEPA 1998). Thus, the need for economical alternative systems that provide efficacious control and maintain product quality throughout processing, storage, and marketing is critical. At present, no single proposed nonchemical method is a suitable substitute for fumigation. In Johnson et al. (1998), we demonstrated the efficacy of an integrated control system for postharvest walnuts that applied an initial disinfestation treatment to incoming products, followed by long-term protective measures during storage.

Any postharvest control system for dried fruits and nuts must be targeted against several species of pyralid moths. In particular, Indianmeal moth, *Plodia interpunctella* (Hübner), navel orangeworm, *Amyelois transitella* (Walker), and raisin moth, *Cadra figuliella* Gregson, are the most economically important postharvest pests of these products in California. Because infestations of navel orangeworm and raisin moth originate in the field and are carried into storage, where adults do not normally reproduce (Simmons and Nelson 1975), initial disinfestation of incoming product is sufficient to reduce damage by these pests. In contrast, Indianmeal moth attacks the product after harvest, and is capable of repeated infestation during storage (Simmons and Nelson 1975), so that long-term protective treatments provide the most efficient control.

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Because none of these pests are considered to be of quarantine significance, the objective of any proposed control strategy would be to maintain acceptable product quality standards. As such, stringent quarantine security treatment efficacy levels are not required.

In this article, we evaluate an integrated method that combines initial disinfestation using controlled atmospheres (0.4% O_2) with protective treatments of a microbial agent (Indianmeal moth granulosis virus), cold storage (≤10°C) or maintenance levels of controlled atmosphere (5% O_2) to control postharvest insect infestations in dried fruit and nut storages. Our study demonstrates the efficacy of the proposed combination treatments for almonds and raisins.

Materials and Methods

Experimental Design. The two-stage experimental design was similar to that used in Johnson et al. (1998). After an initial disinfestation treatment (0.4% O_2 at 25°C for 6 d after a 2 or 4 d purge), three protective treatments and an untreated control were compared. Test species used during the initial disinfestation treatments were navel orangeworm and raisin moth for almonds and raisins, respectively. The protective treatments were a controlled atmosphere of 5% oxygen, cold storage at ≤10°C, and Indianmeal moth granulosis virus (IMMGV) applied as an aqueous spray (57.3 mg virus/kg almonds, 28.7 mg virus/kg raisins). Indianmeal moth was the test insect during the protective treatments for both almonds and raisins.

Sixteen commercial raisin bins (1.3 by 1.3 by 0.65 m) filled with product were used for each test. Each bin held ≈454 kg of raisins or 227 kg of almonds. After the initial disinfestation treatment, four bins of product were isolated in each of four separate rooms for subsequent evaluation of protective treatments, resulting in ≈1,814 kg of raisins or 907 kg of almonds in each treatment room. Experimental rooms for the controlled atmosphere, IMMGV, and untreated control were specifically built for these tests, measured 3 m square by 2.4 m high (21.5 m^3) and were equipped with heating, air conditioning, and ports for introduction of test insects. A refrigerated, insulated cargo container (6 by 2.4 by 2.4 m), also equipped with access ports, was used for the cold storage. The complete test was done twice for almonds and once for raisins.

Initial Disinfestation Treatment. The disinfestation treatment for the single raisin test was begun on 9 October 1995. Disinfestation treatments for the first and second almond tests were begun on 12 November 1996, and 18 August 1997, respectively. Unfumigated product was used for the raisin test and the second almond tests. Only product fumigated with methyl bromide was available for the first almond test.

The controlled atmosphere room had pressure relief valves, a standard air expansion bag, and was sealed to a pressure half-life of 1 min after being pressurized to 25 mm H_2O. A hollow fiber membrane gas separation system (Prism Alpha Nitrogen System CPA-5, Permea, St. Louis, MO) was used to produce the required low oxygen atmosphere. Oxygen levels were monitored with a Servomex 570 paramagnetic oxygen analyzer (Servomex, Norwood, MA). For the raisin test and the first almond test, only one of the treatment rooms was modified to hold controlled atmospheres. For the second almond test, a second room had been modified as described above, and was available for controlled atmosphere treatments.

All 16 bins of product used in subsequent protective treatment studies were first subjected to the appropriate initial disinfestation treatment. For both almond tests, the initial disinfestation treatment was 0.4% O_2 at 25°C for 6 d. This treatment schedule was shown to be efficacious against the most resistant stage of the navel orangeworm in earlier studies with walnuts (Johnson et al. 1998). For the first almond test, only one treatment room was available. Because only eight bins could be treated at a time, half the bins were treated and moved to the appropriate protective treatment rooms before the remaining nuts were treated. The eight bins were arranged in two rows of two bins each, and stacked two bins high. The purge time for the first almond test was 2 d. For the second almond test, two treatment rooms were available, allowing all 16 bins to be treated at the same time. Because simultaneous use of two rooms doubled the air space to be treated by the existing equipment, purge time was lengthened to 4 d for the second almond test.

Initial disinfestation for the single raisin test was targeted against raisin moth. Because preliminary laboratory studies (D.G.B., unpublished data.) found the response of raisin moth to low oxygen atmospheres to be similar to navel orangeworm, the initial disinfestation treatment for raisins was identical to almonds (0.4% O_2 at 25°C for 6 d). Raisins were treated eight bins at a time in a single room, with a purge time of 2 d.

Raisin moth and navel orangeworm used to evaluate the initial disinfestation controlled atmosphere treatment were from laboratory colonies maintained on a wheat bran diet (Tebbets et al. 1978) at 27°C, 60% RH and a photoperiod of 14:10 (L:D) h. Raisin moth was originally obtained from a raisin-packing house in Kingsburg, CA, in 1967, whereas navel orangeworm was originally obtained in 1966 from the University of California, Berkeley. All test insects were held in glass canning jars (0.48 liter) during treatment. The center lid of each jar was replaced with filter paper overlying copper screen. For disinfestation tests with raisins, 50 raisin moth larvae (21–22 d old; fourth instar) were placed in the jars with 100 ml of wheat bran diet and 100 ml of raisins. Preliminary studies indicated that this was the most resistant stage (D.G.B, unpublished data). Tests with almonds used jars containing 50 navel orangeworm larvae (21 d old; fifth instar) in 200 ml of wheat bran diet.

In all disinfestation tests, jars were buried just below the product surface just before treatment. For almond tests, one jar was placed in each of four of the eight treated bins. During treatment of all 16 bins, a jar was placed at each of the eight bin locations, for a total of 400 treated larvae. Four untreated jars, for a total of

200 larvae, were used as controls. The design was similar for the raisin test, except two jars were placed at each bin location and eight untreated jars were used for a total of 800 treated larvae and 400 control larvae. After treatment, jars were brought back to the laboratory and held for adult emergence.

To determine the insect species present naturally in raw product, immediately after each disinfestation treatment the floor of each treatment room was swept and the sweepings examined for insects.

Protective Treatments. Within 1 wk after the initial controlled atmosphere disinfestation treatment, product was moved to the appropriate room and the protective treatments were begun. We used the hollow fiber gas separation system previously described to maintain an O_2 level of 5% in the controlled atmosphere treatment room. Target air temperatures in the cold storage treatment were ≤10°C.

The stock IMMGV preparation used in the protective treatment was produced as a powder (Vail 1991). Bioassay of the stock showed that the LC_{50} was 0.1 μg/g of diet (95% CL = 0.13 (g/g; slope = 1.06 ± 0.05) as estimated by probit analysis (POLO-PC, LeOra Software 1994). For the raisins, a dose of 28.7 mg/kg of product, equivalent to the upper 95% confidence limit of the estimated LD_{99} for the above IMMGV preparation, was selected for application. This dose was equivalent to that used on walnuts (Johnson et al. 1998). Because of the porosity of the almond shell, the applied dose was increased to 57.3 mg/kg of almonds.

We applied 13.0 g of IMMGV dry preparation in 2.5 liter of water per 453.6 kg of raisins and 226.8 kg of almonds (one bin of product). Product was spread on a conveyor belt in a thin layer. The aqueous spray was applied to the product through two TX8 nozzles at 40 psi. The nozzles were placed over the conveyor belt at a height that provided coverage to product moving down the belt.

Temperatures in the controlled atmosphere, IMMGV, and control rooms were kept at 25 ± 2°C. Relative humidity in the cold room was maintained at 60–80% with a low temperature dehumidifier (Ebco, Columbus, OH). We did not attempt to control relative humidity in either of the remaining treatment rooms or in the control room. To ensure that air temperatures were properly maintained, temperature and relative humidity in the treatment rooms were recorded, either with individual dataloggers (Datapod DP-220s, Omnidata, Logan, UT) or a centralized data acquisition system (Campbell, Logan, UT). In each room, sensors measured the air temperature and product temperature 10 cm beneath the surface. In addition, temperatures in the product bins of the cold storage treatment were recorded with thermocouples of 36-gauge copper-constantan attached to a Polycorder datalogger (Omnidata). Two thermocouples were placed in each bin; one at the center of the product and one ≈5 cm below the product surface. Another thermocouple was placed at the center of the cargo container ≈0.3 m above the bins. Temperatures were recorded every 15 min.

Indianmeal moths used to evaluate the protective treatments were from a laboratory colony originally obtained from a walnut packinghouse in Modesto, CA, in November 1967 and were maintained on wheat bran diet. Rearing conditions were 27°C, 60% RH, and a photoperiod of 14:10 (L:D) h. Mated pairs of Indianmeal moth adults were added to the treatment rooms through small access ports (5 cm diameter) as described in Johnson et al. (1998). Five mated pairs of Indianmeal moth were added each week to the almonds, and 15 mated pairs were added each week to the raisins. We added moths to all rooms as soon as the controlled atmosphere treatment room reached 5% O_2, and then once each week until 2 wk before the test was to be completed. To serve as external controls and to estimate the potential number of eggs deposited in the rooms, each week an additional five pairs of moths were placed into 1-liter containers with 250 g of diet and held at normal rearing conditions. All adult progeny produced in these containers were removed and counted.

Based on our earlier walnut studies, we set the duration of the protective treatments in almonds at 17 wk. Unfortunately, a technical problem resulted in the premature termination of the first almond test at 13 wk. The second almond test was terminated at 17 wk as planned. Because Indianmeal moth develops much more slowly on dried fruit (Johnson et al. 1995), we lengthened the duration of the protective treatment on raisins to 41 wk.

Pheromone Monitoring. During the protective treatments, all four rooms were monitored continuously with Pherocon 1C sticky traps (Trece); each trap was baited with Indianmeal moth pheromone lure (Consep Membranes, Bend, OR). We placed one trap in each room ≈6 feet above the floor. Trapped moths were counted each week for each test in the control, IMMGV and cold storage rooms. Trap bottoms were changed when needed. New lures were applied about every 6 wk. Because the door to the controlled atmosphere room was sealed during treatment to maintain treatment atmosphere, moths were counted in this trap only after the room was aerated at the end of each test. All other trap data are reported as weekly counts of Indianmeal moth males. Lures were not replaced in the controlled atmosphere treatment room during either almond test. For the raisin test, lures were replaced by pulling the trap to an access porthole by means of a string.

Product Sampling: Raisins. During the raisin test, we took product samples immediately after the initial disinfestation treatment (0 wk) and then every 5 wk for up to 40 wk from the control, IMMGV, and cold storage treatments. Samples were taken from the controlled atmosphere treatment at 0 and 41 wk, because the door seal could not be broken. A single 3.5–4.0 kg sample was taken from each of the four bins within a treatment room. Raisins were taken from each corner and the center of the bin and placed in paper bags. Samples were frozen for 3–4 d to kill any live insects, allowed to thaw, and sent to an independent laboratory for quality evaluation according to industry stan-

dards. The parameters used in the industry quality evaluation included moisture content, and percentage damage due to mold, insects and other factors.

A second 2.5–3.0 kg sample was taken in a similar manner from each bin and placed in a 7.6-liter bucket. This sample was mixed thoroughly by multiple passes through a sample splitter. We divided the sample in half on the fourth pass through the sample splitter. One half (≈1.5 kg) was placed in a 3.8-liter glass screw-top jar. The metal jar lids were modified by cutting a 6.5-cm hole in the center and soldering metal screen over the opening. Samples were held at 25°C for 2 wk, and then 100 g of wheat bran diet (Tebbets et al. 1978) was added over the top of the raisins to accelerate development of any immature Indianmeal moths present within the samples. Samples were held for another 4 wk and then examined for the presence of adult moths.

The remaining half of the sample was evaluated by weighing out 1 kg of raisins into a large porcelain tray. Raisins were examined individually for insect and other damage, and for the presence of live or dead insects. Damage was recorded as minor insect damage (does not impair the marketability of the raisin) moderate/severe insect damage (marketability of the raisin is reduced), mechanical damage, mold, and other damage. The count and weight of damaged raisins were recorded and the percentage of damaged raisins calculated by weight for each category.

Additional large samples were taken at the end of the raisin test (41 wk) to determine potential survival after treatment. About 1.4 kg of raisins was taken from the surface of each corner and the center of each bin, for a total of 20 samples per room. Raisins were placed in 2-liter plastic buckets closed with organdy cloth held in place by snap-on plastic lids with a 75-mm-diameter hole cut in the center. We added ≈100 g of wheat bran diet to the raisins to speed the development of any Indianmeal moth that might be present. The raisins were held at 25°C for 6 wk, at which time they were examined for the presence of Indianmeal moth adults.

Product Sampling: Almonds. Almond samples were taken from the control, IMMGV, and cold storage treatments at 0, 4, 8 and 12 wk for the first test and at 0, 4, 8, 12 and 16 wk for the second test. Samples were taken from the controlled atmosphere room at 0 and 13 wk in the first test and at 0 and 17 wk for the second test. A single 4-kg sample was taken from each bin by scooping nuts from the corners and the center of the bin. Each sample was thoroughly mixed by multiple passes through a sample splitter. On the final pass through the sample splitter, we divided each sample into halves. One half (≈2 kg) was placed in a paper bag, frozen for 3- 4 d, thawed, and sent to a commercial laboratory for damage analysis according to industry standards. The second half was split again, one half (≈1 kg) was used for quality evaluation, and the remaining half was held at 4°C in reserve or for ancillary tests. We evaluated the almond samples by counting out 500 nuts from each 1-kg subsample and examining them individually for damage and the presence of insects. Damage categories were similar to those for raisins.

Table 1. Survival of raisin moth in raisins and navel orangeworm in almonds after an initial disinfestation treatment of 0.4% O_2 for 6 d at 25°C

Treatment	No. insects treated	Adults emerged	% survival
Raisin test (raisin moth as test insect)			
Control	400	251	63
CA	800	0	0
Almond test 1 (navel orangeworm as test insect)			
Control	200	190	95
CA	400	40	10
Almond test 2 (navel orangeworm as test insect)			
Control	203	203	100
CA	400	0	0

As with the raisins, additional samples were taken at the end of each almond test (13 and 17 wk for the first and second test, respectively) to determine potential survival after treatment. The method used was similar to those for raisins, except that each almond sample was ≈0.9 kg, and no wheat bran diet was added.

Statistical Analyses. For samples taken during the protective treatments and evaluated by laboratory personnel or the commercial laboratory, damage and quality values for each treatment were compared using the SAS general linear model (GLM) analysis of variance (ANOVA) procedure (SAS Institute 1989). With the exception of live Indianmeal moths, an arcsine transformation was done for all parameters to normalize the data. Analyses were done independently for each sample date, with bin samples treated as independent observations within each treatment. Where ANOVA showed significant differences ($P \leq 0.05$), means were separated using Bonferroni *t*-test (SAS Institute 1989). For raisin samples amended with wheat bran diet, Indianmeal moths recovered from the untreated control and IMMGV treatment room were compared using *t*-tests (SAS Institute 1989).

Results

Initial Disinfestation. The results from the initial disinfestation tests against raisin moth and navel orangeworm are given in Table 1. No adult moths emerged from any of the treated insects in either the raisin test or the second almond test. In the first almond test, 10% of the treated insects survived to emergence. Survival of untreated navel orangeworm was high (95 and 100% in the first and second almond test, respectively). The time to emergence of untreated navel orangeworm in the first almond test was about 12 d longer than in the second test. Emergence of untreated raisin moth was lower (63%) than for untreated navel orangeworm.

Sweepings of the rooms after the initial disinfestation treatment of raisins yielded a variety of dead insects, which were assumed to have been present in the product. We found larvae and adults of the dried-fruit beetle, *Carpophilus hemipterus* (L.) and the saw-

toothed grain beetle, *Oryzaephilus surinamensis* (L.), adults of the confused flour beetle, *Tribolium confusum* Jacquelin du Val, larvae of the raisin moth, and a single adult of the Indianmeal moth. Sweepings after the initial disinfestation of almonds recovered larvae and adults of the merchant grain beetle, *O. mercator* (Fauvel), adults of the confused flour beetle, larvae and adults of the navel orangeworm, and larvae of the raisin moth.

Protective Treatments. *Estimation of Indianmeal Moth Egg Deposition.* The mean number (±95% CI) of adult progeny produced per female Indianmeal moth on wheat bran diet under laboratory conditions were 298.8 ± 31.2, 267.0 ± 34.9, and 301.9 ± 21.0 for the raisin, first and second almond test, respectively, for an overall average of 289.2. Given that egg to adult survival for our laboratory isolate under these conditions is ≈95% (Johnson et al. 1995), the estimated number of eggs produced by a single female was 304. For the raisin test, 15 females were added to each treatment room each week for 39 wk, or an estimated 4,567 eggs were deposited each week for a total of 178,107 eggs for the entire test. For the almond tests, five females were added weekly, for an estimated total of 1,522 eggs each week. Moths were added for 11 wk for the first almond test and for 15 wk for the second test, for totals of 16,745 and 22,835, respectively. The total product surface area for the four bins was 5.88 m^2, so weekly egg deposition was estimated to be 777 and 259 eggs/m^2 for the raisin and almond tests, respectively. Total egg deposition for each test was estimated at 30,290, 2,848, and 3,883 eggs/m^2 for the raisin, first and second almond test, respectively.

*Pheromone Traps.*Very few Indianmeal moths were collected in pheromone traps in either the cold storage or controlled atmosphere treatment rooms for any of the three tests. During 41 wk of monitoring the cold storage room in the raisin test, only two Indianmeal moths were recovered. No moths were recovered in the cold storage room during either almond test. In total, 22 moths were recovered in the controlled atmosphere room during the raisin test, two moths were recovered in the first almond test, and one moth was found in the second almond test. In all of these cases, it was most likely that the moths recovered were from those being added each week, and not from infested product.

Weekly pheromone trap counts for each of the three tests in the untreated control room and the IMMGV treatment room are given in Figs. 1–2. In total, 436, 54, and 218 moths were recovered from the IMMGV treatment room in the raisin, first and second almond tests, respectively. Weekly trap catch during the raisin test increased beyond the expected level of recovery of added moths after 13 wk in the raisin test, 7 wk in the first almond test, and 9 wk in the second almond test. After 31 wk in the raisin test, we consistently recovered the braconid parasitoid *Habrobracon hebetor* Say (Hymenoptera: Braconidae) from pheromone traps in the untreated control room. We believe *H. hebetor*, a common parasitoid of stored product pyralids, was largely responsible for the drop in weekly trap catch numbers in the untreated control room.

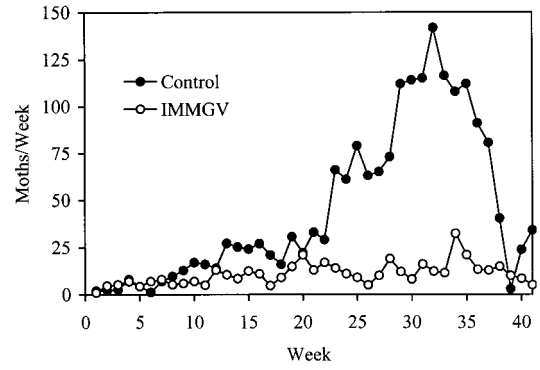

Fig. 1. Number of Indianmeal moths caught each week in the control and IMMGV treatment rooms during the raisin test. Twenty-two moths were caught in the controlled atmosphere treatment room, two were caught in the low-temperature treatment room.

Product Samples: Raisins. The number of live insects and percentage damage found in raisin samples are shown in Table 2. Although we made distinctions during evaluations between minor and serious damage due to Indianmeal moth, damage levels were so low we combined the damage data in a single category for our final analysis. We found no significant difference ($P \geq 0.05$) in the number of live Indianmeal moth between treatments on any of the sample dates. No live Indianmeal moths were found in any of the samples taken from the cold storage or the controlled atmosphere treatment. We detected no damage due to Indianmeal moth in any of the raisin samples taken during weeks 0–15. During weeks 20–40, damage due to Indianmeal moth was found in all samples from the untreated control room, but none was found in samples from the treatment rooms. Damage due to Indianmeal moth in the control samples was highly variable in the final five sample dates, and was significant at 20 ($F = 6.39$; df =

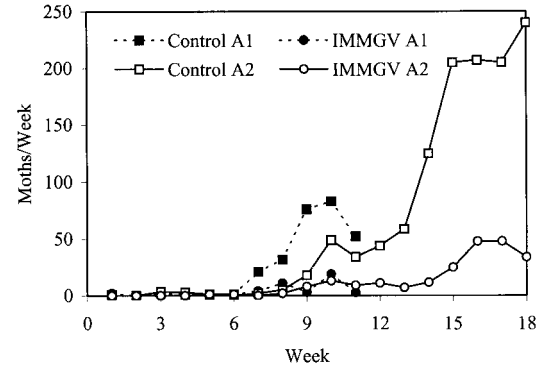

Fig. 2. Number of Indianmeal moths caught each week in the control and IMMGV treatment rooms during the first (A1) and second (A2) almond test. Two moths were caught in the controlled atmosphere treatment room, none were caught in the low-temperature treatment room.

Table 2. Mean (± SD) insects and damage found in raisin samples

Week	Live IMM				% IMM Damage			
	Control	CA[a]	IMMGV	Cold	Control	CA[a]	IMMGV	Cold
0	0	0	0	0	0	0	0	0
5	0	—	0	0	0	—	0	0
10	0a	—	0.25 ± 0.5a	0a	0	—	0	0
15	1.3 ± 1.9a	—	0a	0a	0	—	0	0
20	0.5 ± 0.5a	—	0a	0a	3.2 ± 1.7a	—	0b	0b
25	1.5 ± 0.6a	—	0a	0a	1.8 ± 3.5a	—	0a	0a
30	2.2 ± 1.5a	—	0a	0a	24.0 ± 14.6a	—	0b	0b
35	6.8 ± 6.1a	—	3.8 ± 2.8a	0a	15.5 ± 13.4a	—	0a	0a
40	3.2 ± 2.9a	0a	0a	0a	13.2 ± 5.4a	0b	0b	0b

Among treatments for each variable and sample date, values followed by a different letter are significantly different (Bonferroni t test for mean separation, $P \leq 0.05$).

[a] Samples could not be taken from controlled atmosphere treatment room on certain sample dates because door seals could not be broken.

5, 6; $P = 0.021$), 30 ($F = 5.13$; df = 5, 6; $P = 0.030$), and 40 wk ($F = 12.37$; df = 6, 9; $P = 0.0007$).

The number of live Indianmeal moth recovered from raisin samples amended with wheat bran diet and held for 6 wk is given in Table 3. Data for samples taken from either the controlled atmosphere or the cold storage treatment room were not included in the analysis, because no Indianmeal moths were ever recovered. Only very low numbers were found in the IMMGV treatment room. The numbers recovered from the untreated control samples were significantly higher ($P \leq 0.05$) than those found in the IMMGV treatment room at 20 ($t = 3.31$, df = 3.24, $P = 0.0405$), 25 ($t = 3.94$, df = 3.02, $P = 0.0288$), 30 ($t = 3.80$, df = 3, $P = 0.0319$) and 40 wk ($t = 4.56$, df = 3, $P = 0.0198$). Significantly more ($P \leq 0.1$) Indianmeal moths were also recovered from the untreated control samples at week 35 ($t = 2.55$, df = 3.01, $P = 0.0835$).

A significant number ($F = 23.23$; df = 3, 76; $P \leq 0.0001$) of Indianmeal moths were recovered from the large posttreatment raisin samples taken from the untreated control room (Table 4). In contrast, no Indianmeal moths were recovered from samples taken in any of the treatment rooms.

Product Samples: Almonds. The number of live Indianmeal moth and percentage damage found in both almond tests are shown in Table 5. No live Indianmeal moth or damage due to Indianmeal moth feeding was found in any of the samples from either test at 0 wk. No live Indianmeal moths were found in any of the samples on any sample date from either the controlled atmosphere or cold storage treatment rooms. We found no Indianmeal moth damage in any of the samples from the controlled atmosphere treatment room. In samples from the cold storage treatment room, we found an insignificant level of serious Indianmeal moth damage in the 16-wk sample, but no other Indianmeal moth damage was found.

Samples from the IMMGV treatment room during the first almond test had small numbers of live Indianmeal moth and/or low levels of Indianmeal moth damage for all sample dates, but these were not significantly different from the other protective treatments. During the second almond test, levels of live Indianmeal moth and Indianmeal moth damage in samples from the IMMGV treatment room were not significantly different from the other protective treatments for all but the last sample (16 wk). On the 16-wk sample date, samples from the IMMGV treatment room had significantly more minor damage ($F = 45.02$; df = 3, 12; $P \leq 0.0001$) than the other two protective treatments, and significantly more serious damage ($F = 124.89$; df = 3, 12, $P \leq 0.0001$) than the controlled atmosphere treatment.

The number of live Indianmeal moths in the untreated control room was significantly higher than all protective treatments in the 12 wk samples of the first ($F = 69.08$; df = 3, 12; $P \leq 0.0001$) and second ($F = 11.62$; df = 2, 9; $P = 0.0032$) almond test, and in the 16-wk samples of the second test ($F = 11.04$; df = 3, 12; $P = 0.0009$). In particular, the number of live Indianmeal moth found in the untreated control samples on the final sample date of each test was >100

Table 3. Mean (± SD) live indianmeal moth emerging from raisin samples ammended with wheat bran diet and held for 6 weeks

Sample Week	Control	IMMGV
0	0	0
5	2.3 ± 3.9	0
10	0.7 ± 0.9	0.3 ± 0.5
15	2.7 ± 3.2	0.3 ± 0.5
20	4.5 ± 2.5**	0.3 ± 0.5**
25	17.3 ± 8.6**	0.3 ± 0.5**
30	45.7 ± 24.1**	0**
35	33.5 ± 25.8*	0.5 ± 1.0*
40	31.7 ± 13.9**	0**

*, Treatment means are significantly different (t test, $P \leq 0.1$). **, Treatment means are significantly different (t test, $P \leq 0.05$).

Table 4. Number of live Indianmeal moths found in posttreatment samples of raisins and in-shell almonds (mean ± SD)

Treatment	Raisins	Almond 1	Almond 2
Control	31.4 ± 29.0a	115.6 ± 52.5a	232.0 ± 103.0a
CA	0b	0.1 ± 0.4b	0b
IMMGV	0b	1.8 ± 1.6b	4.4 ± 4.3b
Cold	0b	0.3 ± 0.5b	0.1 ± 0.3b

Among treatments for each commodity, values followed by a different letter are significantly different (Bonferroni t test for mean separation, $P \leq 0.05$).

Table 5. Mean (± SD) insects and damage found in in-shell almond samples

Week	Live IMM				% Minor IMM Damage				% Major IMM Damage			
	Control	CA[a]	IMMGV	Cold	Control	CA[a]	IMMGV	Cold	Control	CA	IMMGV	Cold
						Test 1						
0	0	0	0	0	0	0	0	0	0	0	0	0
4	0.3 ± 0.5a	—	0.3 ± 0.5a	0a	0.1 ± 0.2a	—	0a	0a	0.1 ± 0.2a	—	0.1 ± 0.1a	0a
8	11.7 ± 10.2a	—	0a	0a	1.6 ± 0.4a	—	0.1 ± 0.2b	0b	0.4 ± 0.4a	—	0.1 ± 0.1a	0a
12	109.3 ± 23.2a	0b	1.0 ± 0.8b	0b	6.9 ± 2.5a	0c	1.7 ± 1.1b	0c	20.0 ± 8.4a	0b	1.8 ± 1.2b	0b
						Test 2						
0	0	0	0	0	0	0	0	0	0	0	0	0
4	0a	—	0.3 ± 0.5a	0a	0.1 ± 0.2a	—	0.2 ± 0.3a	0a	0.1 ± 0.3a	—	0a	0a
8	13.7 ± 12.2a	—	1.3 ± 1.5a	0a	0.7 ± 0.9a	—	0a	0a	2.4 ± 2.7a	—	0.4 ± 0.5ab	0b
12	34.0 ± 19.8a	—	0.3 ± 0.5b	0b	1.9 ± 0.7a	—	0.3 ± 0.6b	0b	7.3 ± 5.4a	—	0.7 ± 0.4b	0b
16	482.5 ± 289.7a	0b	4.0 ± 2.2b	0b	4.4 ± 1.0a	0c	0.7 ± 0.7b	0c	28.0 ± 6.3a	0c	2.0 ± 1.2b	0.3 ± 0.4bc

Among treatments for each variable and sample date, values followed by a different letter are significantly different (Bonferroni *t* test for mean separation, $P \leq 0.05$).

[a] Samples could not be taken from controlled atmosphere treatment room on certain sample dates because door seals could not be broken.

times greater than any of the treatments. Minor damage in control samples was significantly higher ($F = 57.51$; df = 3, 12; $P (\leq 0.0001)$ on the 12-wk sample date in the first test, and on the 12-wk ($F = 18.04$; df = 2, 9; $P = 0.0007$) and 16-wk ($F = 45.02$; df = 3, 12; $P \leq 0.0001$) sample dates of the second test. Serious damage in the control samples was also significantly greater on the 12-wk sample of the first test ($F = 54.82$; df = 3, 12; $P \leq 0.0001$), and in the 12-wk ($F = 17.42$; df = 2, 9; $P = 0.0008$) and 16-wk ($F = 124.89$; df = 3, 12; $P \leq 0.0001$) of the second test.

Significantly more Indianmeal moths were recovered from the large posttreatment samples taken from the untreated control room in either the first ($F = 95.5$; df = 3, 76; $P \leq 0.0001$) or the second ($F = 100.1$; df = 3, 76; $P \leq 0.0001$) almond test (Table 4). Samples from all treatment rooms produced very few moths (<5).

Industry Standard Quality Analysis. Results from the industry standard commercial evaluation for raisins is given in Table 6. The industry analysis did not distinguish between Indianmeal moth and other insect damage. Because results changed very little between sample dates, only results from 0, 20, and 40 wk are shown. Mold and insect damage levels were not significantly different ($P \leq 0.05$) between treatments in any of the sample dates. Total damage level in the untreated control was significantly less than damage in the treatments in 20 wk ($F = 6.18$; df = 5, 6; $P = 0.023$), but no significant differences were found in week 0 and 40. Moisture content in samples from the cold storage treatment room was significantly higher in 20 ($F = 8.60$; df = 5, 6; $P = 0.0104$) and 40 wk ($F = 10.67$; df = 6, 9; $P = 0.0012$) samples. The high moisture content of the cold storage samples was due to high humidity levels in the cold storage unit. Humidity levels in the cold storage unit increased as outside temperatures increased, due to poor door seals that allowed leakage of warm air into the unit.

The results from the industry standard commercial evaluation for almonds are given in Table 7. As with the raisin samples, the industry analysis did not distinguish between Indianmeal moth damage and damage due to other insects. By the end of the first test (week 12) samples from the untreated controls had

Table 6. Commercial evaluation of raisin quality parameters (mean ± SD)

Treatment	% moisture	% mold	% insect damage	% total damage
		Week 0		
Control	10.3 ± 0.75a	1.58 ± 0.37a	0.85 ± 0.19a	0.85 ± 0.19a
CA	10.6 ± 1.02a	2.18 ± 0.50a	0.45 ± 0.34a	0.45 ± 0.34a
IMMGV	9.9 ± 0.57a	1.78 ± 0.54a	0.92 ± 0.36a	0.92 ± 0.36a
Cold	11.6 ± 0.99a	1.68 ± 0.61a	0.88 ± 0.17a	0.88 ± 0.17a
		Week 20[a]		
Control	10.4 ± 0.35b	1.52 ± 0.62a	0.55 ± 0.17a	1.15 ± 0.26b
IMMGV	10.0 ± 0.10b	1.75 ± 0.65a	0.38 ± 0.48a	2.00 ± 0.43a
Cold	13.2 ± 1.39a	2.52 ± 0.46a	0.72 ± 0.10a	1.95 ± 0.17a
		Week 40		
Control	11.9 ± 2.47b	1.95 ± 0.50a	1.70 ± 0.34a	1.85 ± 0.21a
CA	10.2 ± 0.43b	1.75 ± 0.87a	1.50 ± 0.64a	1.75 ± 0.48a
IMMGV	10.5 ± 0.42b	1.98 ± 0.86a	1.50 ± 0.45a	1.95 ± 0.58a
Cold	17.5 ± 0.99a	1.95 ± 1.05a	2.18 ± 0.89a	2.75 ± 1.38a

Among treatments for each variable and sample date, values followed by a different letter are significantly different (Bonferroni *t* test for mean separation, $P \leq 0.05$).

[a] Samples could not be taken from controlled atmosphere treatment room because door seals could not be broken.

Table 7. Commercial evaluation of almond quality parameters (mean ± SD)

Treatment	Almond test 1		Almond test 2	
	Insect damage	Total damage	Insect damage	Total damage
		Week 0		
Control	0.5 ± 0.4a	10.3 ± 2.4a	1.0 ± 0.5a	2.9 ± 0.5a
CA	0.4 ± 0.4a	7.6 ± 0.7ab	0.8 ± 1.0a	3.3 ± 0.3a
IMMGV	0.5 ± 0.6a	5.5 ± 1.2b	—[a]	—
Cold	0.4 ± 0.3a	6.8 ± 0.4b	0.4 ± 0.3a	3.0 ± 0.3a
		Week 12		
Control	21.9 ± 7.9a	25.1 ± 7.7a	4.1 ± 1.8a	5.0 ± 1.7a
CA	0.1 ± 0.1b	2.2 ± 1.5b	—[b]	—
IMMGV	0.6 ± 0.6b	2.8 ± 0.8b	1.1 ± 0.4b	2.2 ± 0.6b
Cold	0.4 ± 0.2b	1.9 ± 1.0b	1.1 ± 0.6b	2.0 ± 0.8b
		Week 16		
Control	—	—	22.0 ± 8.1a	22.7 ± 7.7a
CA	—	—	1.1 ± 0.1b	2.9 ± 0.6b
IMMGV	—	—	1.5 ± 0.7b	3.1 ± 1.2b
Cold	—	—	1.0 ± 0.2b	2.7 ± 1.3b

Among treatments for each variable and sample date, values followed by a different letter are significantly different (Bonferroni t test for mean separation, $P \leq 0.05$).

[a] Data lost by laboratory.

[b] Samples could not be taken from controlled atmosphere treatment room because door seals could not be broken.

significantly greater insect damage ($F = 64.91$; df = 3, 12; $P \leq 0.0001$) and total damage ($F = 47.22$; df = 3, 12; $P \leq 0.0001$) than in the treatment samples. In the second test, although untreated control samples at 12 wk had significantly more insect damage ($F = 12.18$; df = 2, 9; $P = 0.0028$) and total damage ($F = 10.37$; df = 2, 9; $P = 0.0046$) than the treatment samples, the difference was not as pronounced as in the first test. By the 16-wk sample date of the second test, significant differences in insect damage ($F = 51.91$; df = 3, 12; $P \leq 0.0001$) and total damage ($F = 43.16$; df = 3, 12; $P \leq 0.0001$) between untreated control samples and treatment samples had approached those found in the 12-wk sample of the first test.

Discussion

Our initial disinfestation controlled atmosphere treatment for raisins proved to be completely effective against raisin moth larvae 21–22 d old. However, subsequent laboratory studies (D.G.B., unpublished data) indicate that larvae (24–25 d old; fifth instar) are slightly more tolerant of controlled atmosphere treatment. For this reason, the treatment may have to be extended to 9 d to ensure complete control.

We observed only 90% mortality of navel orangeworm in the first almond test, but 100% mortality in the second almond test. The apparent tolerance seen in the first almond test may have been due to the presence of larvae in some form of diapause. The first almond test was done in mid-November, while the second test was done in mid-August. Although rearing conditions for all test insects were identical, untreated larvae in the first test emerged ≈12 d later than those in the second test.

Our navel orangeworm cultures were reared under constant temperature and light conditions, but to increase successful mating, oviposition cages were placed near windows to receive natural light. We suspect that this practice may result in some of the larvae going into a diapause-like state. In other studies with laboratory cultures of navel orangeworm, we have seen that some larvae will delay pupation for several weeks, even when reared at optimal temperatures and photoperiods, and that this occurs most often during the winter months (J.A.J., unpublished data).

Several species of stored product pyralids diapause as last instar larvae (Cox and Bell 1991), but diapause in navel orangeworm is not well documented. Gal (1978) found that diapause occurred in navel orangeworm larvae reared under a photophase of 10 h or less. Legner (1983) noted delayed emergence in field populations of navel orangeworm, and suggested the occurrence of diapause triggered by several seasonally varying factors.

Because the first almond test was done in November, when daylengths are short, we believe that test insects may have been in a diapause or diapause-like state. The observed 12-d delay in emergence of untreated navel orangeworm in the first test supports the occurrence of larvae in some form of diapause. The tolerance of other Lepidoptera species to controlled atmospheres is known to be affected by the occurrence of diapause. Moffitt and Albano (1972) and Soderstrom et al. (1990) both noted that diapausing larvae of the codling moth, *Cydia pomonella* (L.), are much more tolerant of controlled atmosphere treatments than other stages. The apparent increase in tolerance seen in the first almond test may have been due to the presence of diapausing larvae.

For almonds, the results for the protective treatments were very similar to those for walnuts (Johnson et al. 1998). The large numbers of Indianmeal moths found in pheromone traps, the high damage levels, and high numbers of Indianmeal moth recovered in nut samples in the untreated storage indicate that the

Indianmeal moth population was far larger than that normally found in commercial storages. The fact that all three protective treatments, storage under 5% O_2, storage at 10°C, or application of IMMGV before storage, were able to protect the almonds by keeping moth populations and damage at such low levels under such high pest pressure proves the efficacy of these methods.

Although the number of Indianmeal moths introduced into the raisin treatments were 3 times that used in the almond treatments, Indianmeal moth populations in the raisins never approached the levels seen in the almonds. Earlier studies have shown that Indianmeal moth survival and development is reduced in dried fruits when compared with nuts (Johnson et al. 1995). Recent research (C. Burks, personal communication) indicates that the lack of fungi reduces the survival of neonate Indianmeal moth larvae on raisins. Similarly, Mondy and Corio-Costet (2000) have shown that fungal sterols increased the survival, developmental rate and fecundity of grape berry moth, *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae). This suggests that our use of dry, raw raisins relatively free of fungi, along with activity by the parasitoid *H. hebetor*, may have kept Indianmeal moth populations to a minimum in the untreated control room. Regardless, evidence from both product samples and pheromone traps showed that all three protective treatments were capable of providing significant protection against Indianmeal moth infestation.

Because facilities and practices for the storage of dried fruits and nuts vary within the industry, the availability of several efficacious pest management methods from which processors may choose is advantagous. As discussed in Johnson et al. (1998), each of the three protective methods has advantages and disadvantages. Storage under controlled atmosphere was the most efficacious, but the sealed storage reduced ready access to the product and present worker safety considerations that do not exist for the other methods. Cold storage was nearly as efficacious as controlled atmosphere, provided ready access to product with no worker safety concerns. Cold storage may also maintain high product quality, provided care is taken to keep relative humidities low.

Both controlled atmospheres and cold storage require extensive capital expenditure for equipment, sealing or insulation of existing storages or building of new facilities. Energy requirements for running controlled atmosphere generators or refrigeration units add to the costs. Historically, the costs for these alternatives are normally higher than the cost of methyl bromide fumigation (Soderstrom et al. 1984, Rhodes 1986, Carpenter et al. 2000) but the current cost of fumigation is rising as restrictions to fumigant use are increased. Application of IMMGV is probably the easiest to impliment and least expensive of the three methods, with current estimates making it comparable to methyl bromide fumigation in cost. Although the IMMGV kept Indianmeal moth populations at acceptable levels under very high pest pressure, it was not as effective as either controlled atmosphere or cold storage. However, because the protection is applied to the nuts themselves, and is independent of physical plant configurations, the product remains protected as it moves through the processing chain. Although the IMMGV preparation is not immediately available, registration of a commercial product is expected soon. The success of this method will depend on future availability and the ability of processing plants to apply the virus to bulk quantities of product.

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